--Figure 7 shows alignment of the deduced amino acid sequence of clone number 19 (*Cal-Nt*; SEQ ID NOs:20 and 22) with a related calreticulin protein sequence CRT1 of *A. thaliana* (U66343, Nelson *et al.* 1997; SEQ ID NOs:21 and 23).

B3

A. Alignment of the N-terminus of *cal-Nt* (SEQ ID NO:20)deducted from the nucleotide sequence obtained using the T7 primer. Box: the hydrophobic leader sequence.

B. Alignment of the C-terminus of *cal-Nt* (SEQ ID NO:22) deduced from the nucleotide sequence obtained using the M13(f) primer. Box: the HDEL conserved domain.--

On page 12, please replace the paragraph spanning lines 1-2 with the following paragraph:

B4

--Figure 9A shows the nucleotide sequence of the syr gene (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2);--

On page 12, please replace the paragraph spanning lines 4-7 with the following paragraph:

--Figure 10 shows alignment of the amino acid sequence of SYR-Nt *(Nicotiana tabacum*; SEQ ID NO:2) with KNOLLE-At (*Arabidopsis thaliana*, Lukowitz *et al* 1996; SEQ ID NO:24),

B5

SYNIA-HUM (Bennett, *et al.*, 1992; SEQ ID NO:25) and SYN1B-HUM (accession number R08740; SEQ ID NO:26), two different members of the human syntaxin family, and syntaxin A of Drosophila (SEQ ID NO:27).--

On page 15, please replace the paragraph spanning lines 13-16 with the following paragraph:

--Figure 27 shows sequence motifs required as template for BotN/C.

A. Cleavage site: SYR-Nt (SEQ ID NO:28); syntaxin 1A-DRO (SEQ ID NO:29); syntaxin 1A-RAT, syntaxin 1A-HUM (SEQ ID NO:30); SSO1-yeast (SEQ ID NO:31); SSO2-yeast (SEQ ID NO:32);

B6

B. recognition motifs X1 and X2 of SYR: left face is mainly hydrophobic (34, 41 and 37 for X1; 175, 182 and 178 for X2); right face is mainly negative charged (35, 39 and 36 for X1; 176, 180 and 177 for X2).--

On page 17, please replace the paragraph spanning lines 23-31 with the following paragraph:

--Figure 41 shows hydrophobicity, alignment and expression analysis of theNt-Syr protein. (A) Key features of Nt-Syr include putativeCa2+-binding (EF-hand; SEQ ID NO:33) and nucleotide-binding (NBS; SEQ ID NO:34) sites, partially conserved amphipathic X1 and X2 domains (*) for recognition and cleavage by BotN/C toxin, and three putative coiled-coil domains (H1-H3). High amino acid conservation with Knolle of Arabidopsis thaliana (15; SEQ ID NOs:36 and 39) and with human Syntaxin-1A (Syn1A; SEQ ID NOs:37 and 40) (3) is found in the epimorphin pattern domain (=H3) (Nt-Syr = SEQ ID NO:35; Knolle = SEQ ID NO:36; Syn1A = SEQ ID NO:37) and the C-terminal hydrophobic tail (Nt-Syr = SEQ ID NO:38; Knolle = SEQ ID NO:39; Syn1A = SEQ ID NO:40). The truncated protein (Sp2), corresponding to the first 279 amino acids was N-His-tagged and used for generating antibodies.--

B7

On page 29, please replace the paragraph spanning lines 1-11 with the following paragraph:

--All 20 cDNAs were then analysed by their nucleotide sequence to get an idea which clones might be candidates for ABA signalling elements and which ones could be definitely excluded from further study. All the possible amino acid sequences deduced from this nucleotide sequence were compared with the protein databases. Similarities indicated that some genes were comparable to enzymes or proteins with functions unrelated or unlikely to be associated with signalling, others showed no similarity to anything in the databases, but were considered interesting because they contained several putative membrane spanning regions. Finally, several genes of major interest showed homology with proteins known to have distinct functions in signal transduction. Amongst them were a small G-protein (*smG-Nt*; SEQ ID NO:14), a calreticulin (*cal-Nt*; SEQ ID NO:20), a receptor kinase (*rek-Nt*; SEQ ID NO:6), and a syntaxin like (*syr*) protein (SEQ ID NO:2).--

B8

On page 35, please replace the paragraph spanning lines 2-11 with the following paragraph:

--One of the clones, number 15 (SEQ ID NO:14) codes probably for a Rab/Ypt type small G-protein. Even though it can not possibly be a receptor, it could be involved in the signal pathway more downstream of the initial perception event. Similarity searches were performed with a 109 amino acid sequence of the cDNA (15) isolated from *N. tabacum* (SMG-Nt2; SEQ ID NO:14) (Fig. 6). The highest identity (74.2%) was detected with a constitutively expressed Rab/Ypt -related sequence isolated from soybean (*Glycine max*), GRM1 (SEQ ID NO:15). Other very homologous small G proteins are derived from *Lotus japonicus,* RAB11G (SEQ ID NO:16) or from *Arabidopsis thaliana* (ARA-4; SEQ ID NO:17, and RAB11; SEQ ID NO:18). Additionally, SMG-Nt2 showed 70.2% homology with another small GTP binding protein of *N. tabacum,* NT-RAB11E (SEQ ID NO:19).--

B9

On page 35, please replace the paragraph spanning line 21, through page 36, line 2, with the following paragraph:

--Figure 7 shows an alignment of the clone number 19, *cal-Nt* (SEQ ID NOs:20 and 22) with the *Arabidopsis thaliana* calreticulin (SEQ ID NOs:21 and 23). The alignments of a 178 amino acid stretch at the 5' end resulted in a similarity of 63.1 % and the alignments for 86 amino acids at the 3' end led to 48.8% similarity between the two sequences. Calreticulins are calcium binding proteins in the endoplasmatic reticulum and have an established role as molecular chaperones. It was also suggested they may play a role in signal transduction, specifically in calcium distribution. Some reports support specific functions for calreticulins outside the endoplasmatic reticulum, such as the interactions with steroid hormone receptors. The sequence show a hydrophobic leader sequence, at the N-terminal site of the protein, and a HDEL motif indicating the location in the endoplasmatic reticulum (Fig. 7).--

B10

On page 36, please replace the paragraph spanning lines 4-9 with the following paragraph:

B11

--Clone number 9, *rek-Nt* (SEQ ID NO:6), appeared to be homologous to receptor kinase type proteins, from *Brassica oleracea* (SEQ ID NO:8) or *Brassica campestris* (SEQ ID NO:9) or *Ipomoea trifida* (SEQ ID NO:7). Figure 3 shows the alignment of the two separately sequenced amino acid strands. The similarity between the N-terminal sequences (Fig 3A; SEQ ID NOs:6-9), was found to be higher (approximately 60%) than of the C-termini (Fig 3B; SEQ ID NOs:10-13), which showed around 40% homology with the receptor kinases from other plant species.--

On page 36, please replace the paragraph spanning lines 20-23 with the following paragraph:

B12

--Experimental evidence suggested that clone number 14, *syr* (SEQ ID NO:2) which shows homology to the syntaxin-related gene of *Arabidopsis thaliana* is of particular interest. One of these indications is given by the observation of currents when this protein was expressed in oocytes.--

On page 36, please replace the paragraph spanning line 24 of page 36 through page 37, line 2, with the following paragraph:

B13

--One clone, p*syr* isolated via the sib-selection strategy yielded currents in oocytes when expressed in excess. BlastX searches revealed homology with an *Arabidopsis thaliana* protein KNOLLE (SEQ ID NO:24) which is a syntaxin-like protein. Syntaxin proteins are members of a big family and are thought to function as receptors for transport vesicles with different isoforms of this family localized to various membranes throughout the cell. Syntaxin is Greek meaning "putting together in order".--

On page 40, please replace the paragraph spanning lines 3-20 with the following paragraph:

B14

--Figure 9A shows the nucleotide sequence of the cDNA insert of the *syr* clone (SEQ ID NO:1). The derived amino acid sequence (SEQ ID NO:2) is displayed for the open reading frame starting with an ATG sequence at position 17 and terminating at position 920 with a TGA stop codon. The amino acid sequence is 300 amino acids long. Panel B of Fig. 9 shows the strategy used during sequencing. Two deletions were made in either end of the cDNA insert. In order to delete the 3' end of the gene, the p*syr* was digested with *BglII* and *BamHI* restriction enzymes, and the remaining strand was religated. This new construct, p*syrbb* was used as a template in a sequencing reaction with the M13(f) primer. The other 5' end was deleted by digesting the p*syr* clone with *SacI* and *EcorI*, the overhanging single strand DNA ends were made blunt using the T4 polymerase and the vector was consequently religating. This new construct, p*syrse* was used as template in a sequence reaction with the T7 primer. The centre part of the cDNA between the *BglII* and *SacI* restriction enzymes was cloned into pBluescript vector, so that the insert is flanked by the two M13 primers: the forward M13(f) and the reverse M13(r) primer, which were used in the two sequencing reactions with this construct, p*syr2*. By merging the sequences of these reactions into a contig (Fig.

B14

9B), all dubious nucleotide sequences could be resolved and the full length sequence of the syntaxin related gene was obtained (Fig. 9A).--

On page 40, please replace the paragraph spanning line 22 through page 41, line 3, with the following paragraph:

B15

--The SYR amino acid sequence (SEQ ID NO:2) was aligned with the KNOLLE syntaxin like protein from *Arabidopsis thaliana* (SEQ ID NO:24) and with two proteins from the syntaxin family, syntaxin A (SEQ ID NO:25) and syntaxin B (SEQ ID NO:26) from human (Fig. 10). The protein sequence contains 3 main regions. (1) The hydrophobic C- terminus which can be a membrane spanning domain. (2) A zone upstream from the hydrophobic domain consists of an epimorphin pattern as aligned with the 'boxes' of the Prosite Database (*Lasergene*, DNA*, Madison, USA). (3) The N-terminus of the protein sequence is hydrophilic and does not contain significant homology to known sequence signatures.--

On page 42, please replace the paragraph spanning lines 3-21 with the following paragraph:

B16

--More particularly, sequencing of the transcript cDNA showed that it contained an open-reading frame that encoded a syntaxin- (t-SNARE-) related protein (Nt-Syr) of 300 amino acids with a predicted molecular mass of 34.01 kD and an isoelectric point of 7.95. Alignments of Nt-Syr protein by the Clustal method using Megalign (DNAstar, Madison USA) showed 37.7% identity with one other syntaxin-like protein from plants, the Knolle gene product identified from Arabidopsis thaliana, and a low but significant homology (22.8% amino-acid identity) to its closest mammalian homolog encoded by the human syntaxin gene SYN-1A. Structural and PRO-SITE analyses of Nt-Syr revealed features common to syntaxin proteins (Fig. 41) including a single, putative membrane-spanning (hydrophobic) domain at its C-terminus (Fig. 41; SEQ ID NOS:38-40), and three domains (H1-H3) with high probabilities for forming coiled-coil structures in protein-protein interactions. Of these putative coiled-coil domains the third, adjacent to the putative membrane-spanning region (SEQ ID NO:35) showed 84% identity (92% homology) with the epimorphin consensus sequence of mammalian Syntaxin-1A (SEQ ID NO:37). Nt-Syr also showed partial conservation of the three dispersed sites necessary for binding and cleavage of the protein by Clostridium botulinum type C toxin (BotN/C; Fig. 41). However, unlike other syntaxin proteins characterised to date, Nt-Syr was found to include a single, EF-hand consensus sequence (SEQ ID NO:33) between amino-acid residues 16 and 28, and a putative nucleotide binding site (SEQ ID NO:34) between residues 114 and 119.--

On page 77, please replace the paragraph spanning lines 19-20 with the following paragraph:

B17

--The T7 primer is a 20 mer: 5'-TAATACGACTCACTATAGGG-3' (SEQ ID NO:41)
M13(f) is a sequence primer (17 mer): 5'-GTAAAACGACGGCCAGT-3' (SEQ ID NO:42)--

On page 78, please replace the paragraph spanning line 26 through page 79, line 5 with the following paragraph:

--A cRNA combination of *smG-Nt* (the small G-protein-like) (SEQ ID NO:14) and the *cal-Nt* (the calreticulin-like) (SEQ ID NO:20) together with the *syr* syntaxin-like clone (SEQ ID NO:1) in a relative concentration of 1 (*smG-Nt*) : 1 (*cal-Nt*) : 0.1 (*syr*), was expressed in oocytes. Some current fluctuation associated with to the syntaxin-like clone was detected but no measurable response to ABA was recorded. This was tested twice in different oocyte batches with $n_{total} = 7$. Also when the three unknown membrane proteins of clones number 2, 8 and 12, together with the syntaxin-like protein were coexpressed with a ratio of (1(2):1(8):1(12):0.1(*syr*)) in oocytes, no ABA signal was recovered. This was also tested on two separate occasions with $n_{total} = 4$.--

B18

On page 81, immediately following the table and before "Example 18", please insert the following paragraph:

B19 --Primer M13(f) = SEQ ID NO:42; Primer M13(r) = SEQ ID NO:43--

On page 93, please replace the paragraph spanning line 24 through page 94, line 3, with the following paragraph:

--The first peptide, SP1 consisted of a 13 amino acid long antigenic stretch of the SYR amino acid sequence (SEQ ID NO:2). Because of the small size of the peptide, it was conjugated to a keyhole limpet haemocyanin (KLH). KLH is routinely used as peptide-carrier protein and is hardly antigenic. The peptide conjugate was ordered from Pacemaker (Exeter, UK).

B20 Peptide: KLH - CGPGSSSDRTRTS (SEQ ID NO:44)--

On page 102, please replace the paragraph spanning line 20 through page 103, line 2, with the following paragraph:

--The peptide SP1 was chosen from amino acid 115 to 127 of the SYR protein (SEQ ID NO:2). This region was predicted highly antigenic and hydrophilic and had a high surface probability, as predicted by the PROTEAN program from *Lasergene* (DNA*). A conjugate of this SP1 with the KHL protein (SEQ ID NO:44) was injected into 2 rabbits (Kent University, Canterbury (UKC)). The blood sera, sampled two to three weeks after a first, second and third SP1-KHL injection from either rabbit was tested for its recognition of the peptide SP1-KHL and of the SYR protein expressed in yeast (see below). No reaction was detected neither with SP1-KHL and SYR. Therefore, antibodies were generated against a second peptide.--

B21